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(57) Abstract

The present invention relates to superparamagnetic particles, a way of producing said particles and their use. More specifically, the invention describes a process for fabrication of said particles, said process yielding particles with a high recovery in a simple one step procedure.

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SUPERPARAMAGNETIC PARTICLES. A WAY OF PRODUCING SAID PARTICLES AND THEIR USE.

This invention relates to magnetically responsive superparamagnetic particles, a way of producing said particles and their use.

More specifically the invention describes a method for the production of superparamagnetic particles which are made of a metal oxide surrounded by a surface layer of a biologically acceptable polymer. In this form the particles may be used as a contrast agent in medical diagnostics, in particular in Magnetic Resonance Imaging (MRI). They may also be used as a matrix for biological molecules, thus functioning as a separation device. Another area for their use is by combining said particles with a drug which after injection into the blood stream is stopped in an predetermined area using an external magnet.

The invention is based on magnetically responsive particles which are superparamagnetic, indicating that they are not permanently magnetized when subjected to a magnetic field. This means that the particles, after withdrawing of the magnetic field, are easily resuspended. Using magnetically responsive particles within the areas mentioned above, it is of utmost importance that the particles are superparamagnetic in order to avoid permanent aggregation.

Magnetic particles has for long times been discussed as a extremely effective separation device (Hirschbein et al. Chemtech. March 1982. 172-179). However, prior art regarding magnetic particles lack one or several of the demands needed in order for the technology to obtain general acceptance.

The above mentioned superparamagnetic property is thereby one of the most important.

Ithakissios (US Patent 4,115,534. Sep. 19, 1978) describes magnetic polymer particles in the size range 10-100 µm where the polymer material is covalently crosslinked.

Avrameas & Guesdon (US Patent 4,241,176. Dec. 23, 1980) describes magnetic polymer particles where magnetite has been ntrapped in a polyacrylamide-agarose polymer in an emulsion process. The size of the spheres is 50-500 µm.

Widder et al (US Patent 4.230,685. Oct. 28. 1980) describes a way of producing magnetic particles where the magnetite is entrapped into albumin/protein-A with subsequent covalent stabilization of the polymer mixture.

Ugelstad et al (PCT/NO83/00014) describes a way of producing particles where the magnetic material is precipitated within prefabricated polymer particles of a defined size.

Czerlinski (US Patent 4,454,234. June 12, 1984) describes magnetite particles which has been surface coated with a crosslinked polymer of acrylamide.

Molday (US Patent 4.452,773. Jun.5, 1984) described a way of producing magnetite particles with a size of 10-70 nm, which are surface coated with a dextran polymer. Dextran has a high molecular weight and is not degraded in the body, thus particles produced in this way are less suitable for medical applications. After production these particles are further processed by centrifugation at 28.000 rpm, alternatively the particles are allowed to pass a gel chromatography column in connection with the coupling of affinity ligands. Both of these methods may be used in laboratory scale production, however, the technologies are not suitable in the production of particles to be used in large scale separation of fermentation suspensions or the production of superparamagnetic particles for the use as a contrast agent, as discussed in this invention.

As is discussed in the Molday patent above, the particles may not be separated with conventional magnets. This is also seen in the examples presented, where only examples referring to the separation of cells or cell organells are described. In the separation of cells or cell organells, a large number of magnetic particles are associated to the surface of one cell, whereby the total amount of magnetic material attached to a cell makes it responsive to the magnetic field obtained by conventional cobalt-samarium magnets.

Schröder & Borrebaeck (EPC 83901116.0) describes the entrappment of magnetite particles within a carbohydrate matrix by an emulsion process with subsequent stabilization by crystallization of the carbohydrate polym r.

Chagnon et al (EP 0 125 995) describes a process for the production of magnetic particles in a two step procedure; in the first step the magnetic particles are produced, and in step two, these particles are, after extensive washing, subjected to a surface coating with one or several silicone polymers.

Two steps in the process of Chagnon thus differs significantly from the present invention:

1. On Page 20 is described the ratio between $FeCl_2$ and $FeCl_3$ to be used in order to obtain an optimal recovery. In the text and in the examples, the best particles are achieved using a ratio Fe^{2+}/Fe^{3+} of 2/1 or 4/1. If Chagnon is using a ratio of Fe^{2+}/Fe^{3+} of 1/2 or 1/4, heterogeneous particles which "bleeds", finally being totally dissolved in the washing steps (page 20, line 15-25) is obtained. This is in contrast to our own results where optimal particles are obtained with ratios of Fe^{2+}/Fe^{3+} contradictory to Chagnon.

2. On page 20 is described how the recently prepared particles are washed in an NaCl solution in order to ensure that the final product is resuspendable to particles in the size of interest. However, this is not necessary according to the present invention.

3. On page 35 is described how Chagnon prior to the surface coating of silicone has to suspend the particles by powerful homogenization in order to obtain a final product consisting of particles which upon suspension forms a monodisperse suspension.

This is also an experience from the inventors of the present invention: if you don't perform the precipitation of metal salts to metal oxide in the presence of a protective colloids, such as starch, the ferromagnetic particles obtained have a size of 5-50 µm, making them unusable for the purposes as described in this invention.

Using superparamagnetic particles in the areas, as described in this invention, the size of the particles and the choice of polymer is of great importance.

Within medical diagnostics there is primarily a wish to obtain an contrast agent for the liver and the spleen. This can be accomplished using injection of particles into the blood stream having a size of 0.4-1.0 µm, since particles having this size are eliminated from the blood stream by these organs.

Using superparamagnetic particles within biotechnology separation it is also a wish to have particles in the same size range due to the large area per volume obtained. As an example, reducing the size of a particle from 10 to 0.5 µm, the area accessible for attachment of affinity ligands is enhanced 20 times, as calculated on a defined volume packed spherical particles.

The size should, however, not be below 0.3 µm, since the magnetically

responsive particle thus contains too small amounts of magnetic material to be attracted be a magnetic field. Furthermore, particles having a size below 0.3 µm possess particle/liquid interactions resulting in a suspension behaving more like a magnetic fluid and not as a suspension where the particles easily can be retrieved.

Despite what is known about magnetic separation, as described in the above mentioned patents/patentapplications, the magnetic separation technology has not yet been used in large scale separation, such as in fermentation, since the particles has to be to produced in a simple way in large quantities with the subsequent attachment of affinity ligands. In large scale separation, separation of e.g. human proteins produced by genetically engineered bacteria in fermentation volumes of several thousands of liters is discussed.

In the separation process, under the influence of the magnetic field, the superparamagnetic particles will aggregate, however, due to the superparamagnetic property, the particles will be resuspended as soon as the magnetic field is switched off, thereby allowing for the affinity attached protein to be recovered.

Another item using the present invention is that the structure of the particle (a core of metal oxide surface coated with a polymer) renders a system where the affinity ligand is associated only to the surface of the particle. Thus, the final product is economically advantageous as compared to macroporous particles since the amount of affinity ligand (e.g. a monoclonal antibody) used can be reduced. Furthermore, the surface association of the monoclonal antibody renders rapid adsorption of the substance of interest due to the lack of the diffusion barriers.

Thus, magnetic separation using the particles according to the present invention will give the following advantages:

- the number of separation steps can be reduced
- higher yield
- faster processing

This combination thus leads to reduced costs for the separation using the particles according to the present invention.

By the use of the process for the fabrication of superparamagnetic particles according to the present invention, it is now possible to achieve this.

Thus, the present invention relates to superparamagnetic particles, made of a core of magnetite, surrounded by a pharmacologically acceptable carbohydrate polymer, the superparamagnetic particle having a size of 0.1-2.0 µm produced by precipitation of iron salts dissolved in a starch solution.

The process for fabrication of the superparamagnetic particles according to the present invention is based on optimization of parameters resulting in particles which all fulfills the demands mentioned above. Thus, the process renders a high yield where further secondary purification steps in order to obtain optimal superparamagnetic particles having the adequate size and magnetic properties are not needed.

Thus, the process according the this invention, results in particles in a high yield in a simple one step procedure.

The process is based on the well known technology of precipitating iron salts in alkali, whereby the metal oxide is formed. However, this precipitation is performed in a way that both the starch and the iron salts simultanously are added to the alkali solution (e.g. NaOH) while sonicating. The obtained solution is neutralized to pH 7. The suspension obtained, containing a monodisperse suspension of superparamagnetic particles, having a size of 0.2-2.0 µm, is thereby ready for use.

For the person skilled in the art it is now easy the use the suspension for various purposes. As an example, the suspension, containing a monodispers suspension of superparamagnetic particles in a solution of carbohydrates in an alkali or neutral environment, directly or after a concentration step, can be emulsified in an organic solvent, a crosslinker may added to the emulsion or the water solution of superparamagnetic particles, whereafter these are stabilized into a water-insoluble three dimensional crosslinked superparamagnetic microsphere.

EXAMPLE 1.

2.7 g $FeCl_3 \times 7\ H_2O$, 4.5 g $FeCl_2 \times 4\ H_2O$ and 3.0 g of starch is dissolved in 10 ml of water by gentle heating. This solution is added dropwise to 100 ml of 1.0 M NaOH while sonicating. After adding all of the iron-chloride /starch solution the suspension is sonicated for another 5 minutes whereafter the suspension is neutralized with HCl to pH 7.0.

The size of the obtained monodisperse suspension of superparamagnetic

particles is measured in an Coulter Counter Multisizer with the following result:

Average size: 0.775 µm.

99% of the particles is found in the size range 0.35 - 1.22 µm.

Dry weight: 25 mg/ml, where 85% is magnetite.

Number of particles/ml: 7×10^8

CLAIMS.

1. Superparamagnetic particles, constituting of a core of magnetically responsive material, surrounded by a coating of a biologically acceptable carbohydrate polymer, the particles having a size in the range of 0.1 - 2,0 µm.

2. Superparamagnetic particles according to claim 1, **characterized by,** that the magnetically responsive material consists of a metal oxide of iron, nickel or cobalt.

3. Superparamagnetic particles according to claim 1, **characterized by,** that the biologically acceptable carbohydrate polymer is chosen from the group of starch, pullullan or glycogen.

4. A way of producing superparamagnetic particles according to claim 1-3, **characterized by,** that the particle is produced by precipitation of a solution of a metal salt, dissolved in a water solution of the biologically acceptable carbohydrate polymer, into an alkali solution while having a high energy input into the alkali solution.

5. A way of producing superparamagnetic particles according to claim 1-4, **characterized by,** that the energy input is performed by sonication of the alkali solution.

6. A way of producing superparamagnetic particles according to claim 1-5, **characterized by,** that the alkali solution consists of a solution of sodium hydroxide.

7. Superparamagnetic particles according to claim 1-6, **characterized by,** that the particles upon use is suspended in physiologically acceptable solution.

8. The use of the superparamagnetic particles according to claim 1-7 within medical diagnostics.

9. The use of the superparamagnetic particles according to claim 1-8 within affinity separation.

INTERNATIONAL SEARCH REPORT

International Application No PCT/SE88/00561

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC 4

A 61 K 9/16, 47/00, 49/00; C 07 K 17/00; G 01 N 33/553

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC 4	A 61 K, G 01 N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

SE, NO, DK, FI classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
P,X	WO, A1, 88/00060 (ADVANCED MAGNETICS INCORPORATED) 14 January 1988 See the claims 2-4, 9, 11 & EP, 275285 US, 4770183	1, 2, 7

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
1989-02-15	1989 -02- 20
International Searching Authority	**Signature of Authorized Officer**
Swedish Patent Office	Solveig Gustavsson

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

(partially)

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 8 because they relate to subject matter not required to be searched by this Authority, namely:

in the aspect when the claim concerns diagnostic methods of the human or animal body (see article 17 (2) and rule 39).

2. [ ] Claim numbers ..... , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically

3. [ ] Claim numbers ....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fee.